

Consistent Hatchery and Nursery Yields for Bivalve Molluscs using Health Management and Analysis of Production Systems

Florida Clam Industry Workshop

Cedar Key

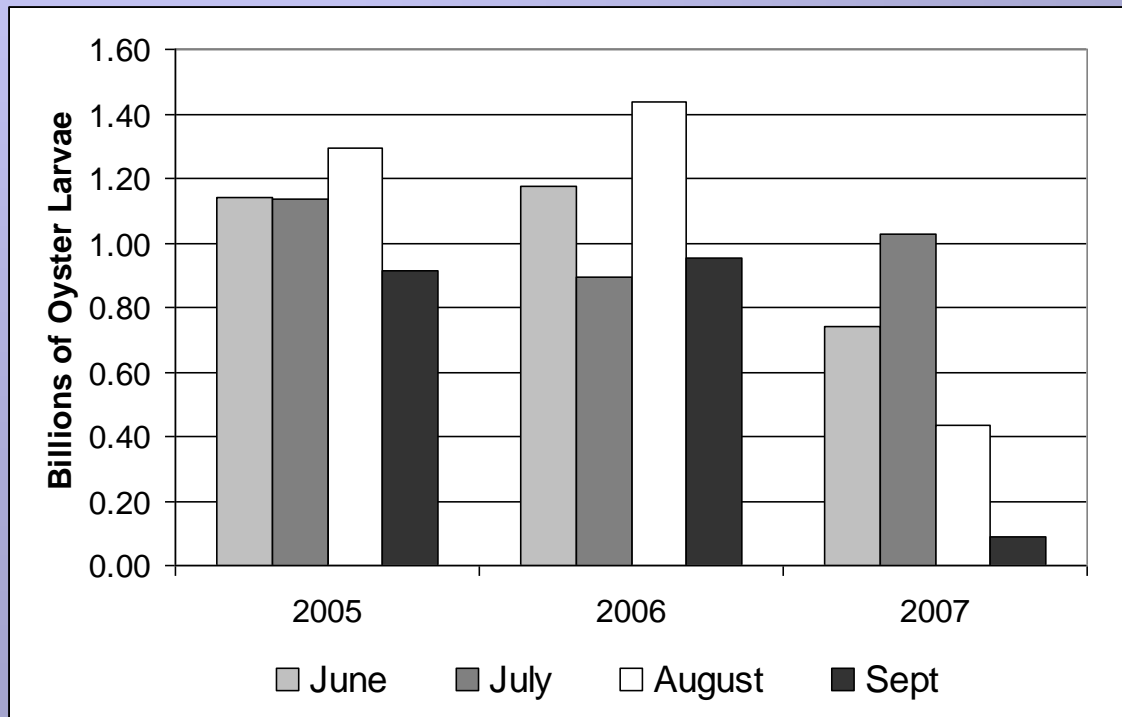
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Hatchery/nursery health management goals.....

- **Predictable production**
- High survival to sale and after sale
- **High growth, health and condition**
- Efficient, profitable production
- **Minimization of waste**
- Compliance with regulatory requirements

Example of consequence of vibriosis, one type of hatchery problem, in one west coast shellfish hatchery.....production drop of 51% plus in 2007...



Shellfish farming has evolved to a vertically integrate farming practice.....

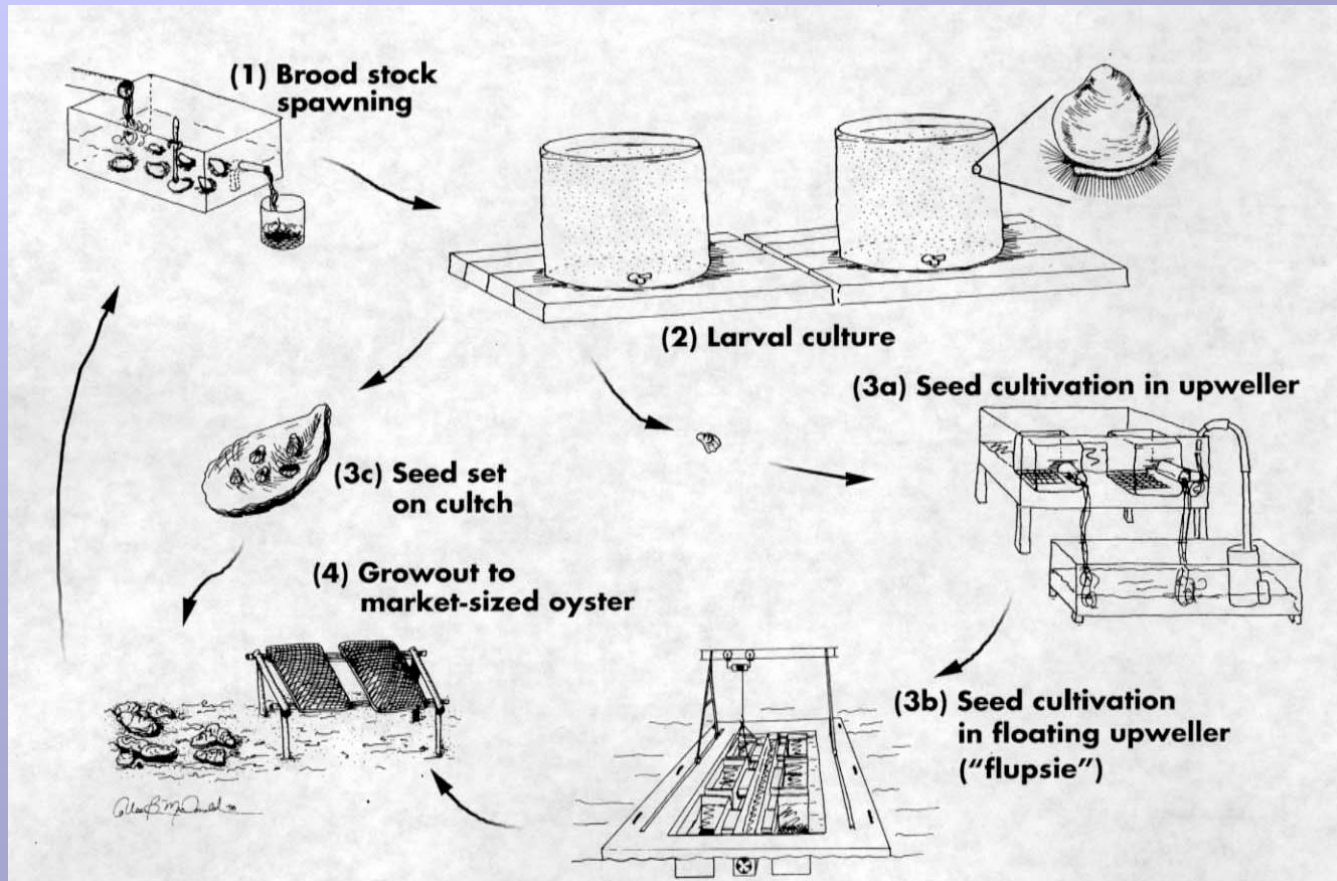


Fig. 2. Cycle of intensive bivalve mollusk husbandry. From Elston (1999).

Health management topics for hard clams.....

- 1. Pathogen contamination, usually bacterial**
- 2. Animal condition assessment**
- 3. Water quality monitoring and management**

Health management has been a key component in the development of all successful forms of animal husbandry. For shellfish intensive culture, we are all “writing the book” as we go, we have made significant progress, but what we don’t know is still much more than what we know.

Health management in hatchery/nursery...

- **Bacterial monitoring and management**
- **Brood stock source, condition and management**
- **Larval and juvenile handling and management**
- **Micro-algal food culture management**
- **Water source(s), quality and management**

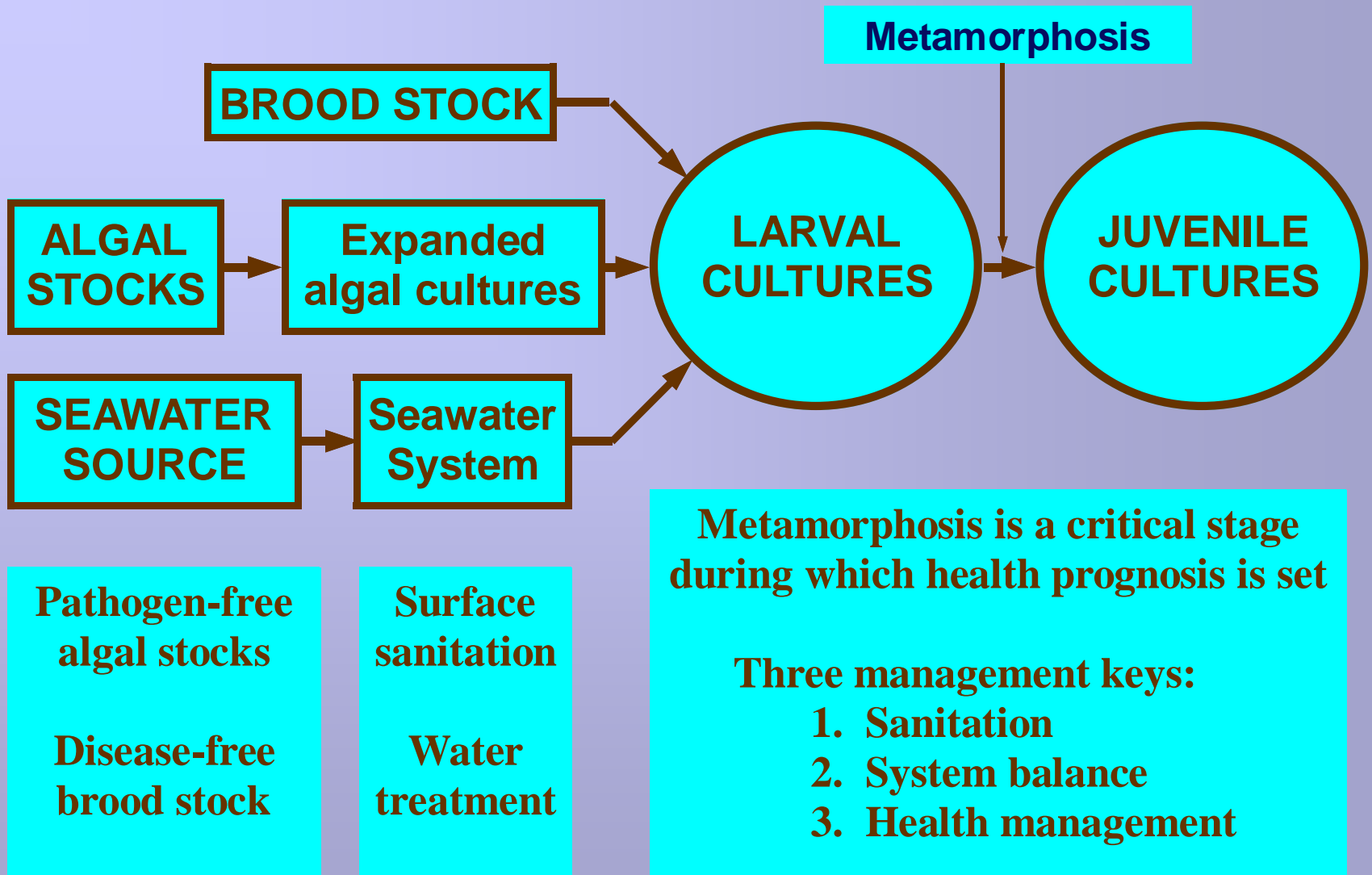
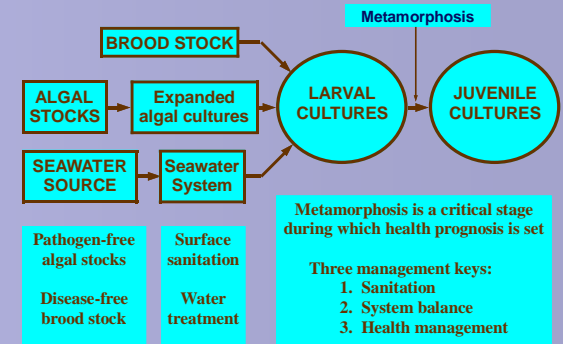


Fig. 1. Schematic diagram of intensive hatchery and nursery production of molluscan shellfish with notes regarding health management. From Elston and War (2003).

Bacteriological management – bacterial colonization, an uncontrolled variable

- Where to sample in the hatchery
 - All locations of input
 - All locations of maintenance of contamination
 - Examples to follow.....
- How to sample in the hatchery
 - Demonstration on sanitation indications – using algology skills
 - Probably need help with more quantitative sampling
- More details in handout and laboratory session

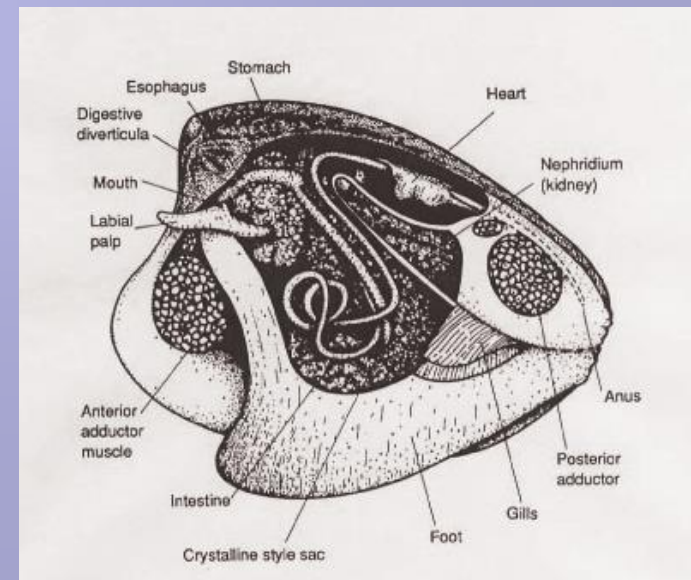
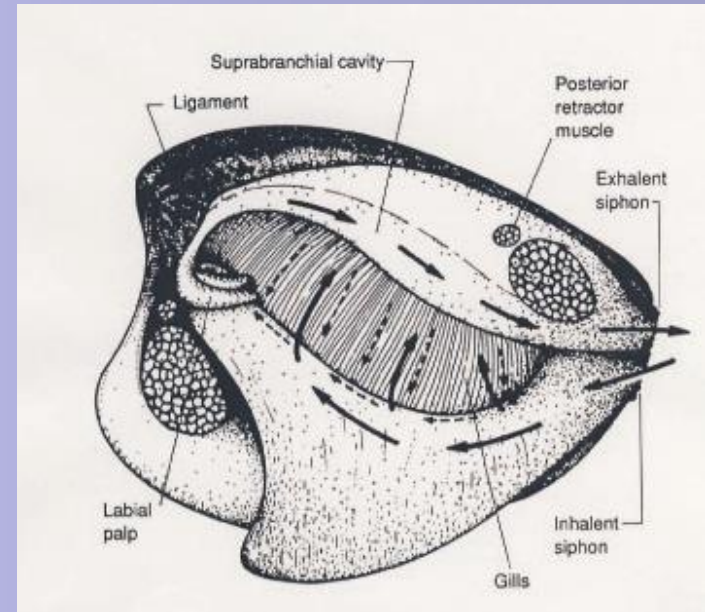


Vibrio locations in hatchery that are high risk for maintenance:

- Bag culture systems
- Tank bottoms of affected larvae
- Brood stock in flow of contaminated water
- Areas with high humidity in the hatchery
- Wet areas that have high air flow, - air coolers and condensation
- Thio-sulfate concentrate that incubates for more than one or two days

Broodstock.....

- Free from reportable diseases, need for a **Shellfish High Health Program** for every shellfish farm
- Condition factor – if naturally conditioned, condition could be variable and contamination possible with bacteria, protozoa
- Hatchery conditioning tanks are a potential source of bacterial contamination buildup
- Contamination can be transferred to eggs and larvae, but generally there is a high degree of dilution



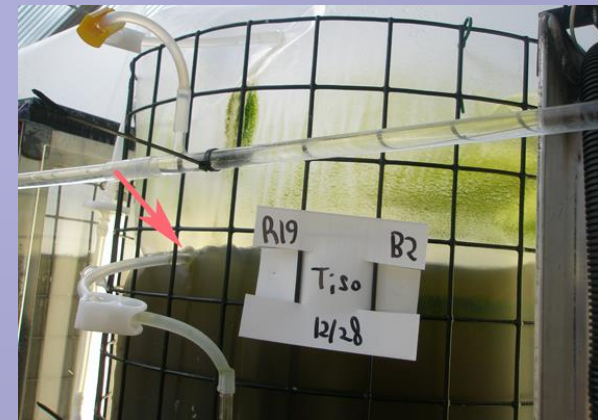
Algal culture, static or continuous is a persistent source of contamination....

Pathogenic bacteria often co-exist with algae and grow to high densities

Result is that toxic brew can be fed to larvae or seed

Solution must start with

- Clean stock cultures
- Uncontaminated water
- Good technique to reduce contamination



Production hatchery examples of contamination with pathogenic vibrios....

Why vibrios?:.....ubiquitous and opportunistic....degrade biological tissue, and certain strains produce toxins and invasive disease

Sample Type	Number of Samples	<i>Vibrio spp.</i> as % composition of total 48 hour plate counts (average) ^a	Median concentration of <i>Vibrio spp.</i>	Maximum observed concentration of <i>Vibrio spp.</i>
Microalgal food stock cultures	12	85%	5.44 x 10 ⁵	2.01x 10 ⁶
Microalgal food static carboy cultures	6	83%	3.52 x 10 ⁵	6.72x 10 ⁵
Microalgal food vertical continuous flow bag cultures	38	49%	2.60 x 10 ⁴	1.32 x 10 ⁶
Microalgal food horizontal continuous flow bag cultures	13	66%	3.60 x 10 ⁴	6.00 x 10 ⁵
Microalgal food static tank cultures (20L to 25,000 L volume)	31	34%	7.20 x 10 ³	3.92 x 10 ⁵
Larval tank water	22	35%	1.06 x 10 ³	3.28 x 10 ⁴
Geoduck clam brood stock water	14	46%	2.40 x 10 ²	1.00 x 10 ³

and sodium thiosulfate – a haven for pathogenic vibrio bacteria

Pathogenic vibrio contamination in hatchery air supplies and algal culture rooms.....

Sample Type	<i>V. tubiashii</i> (cfu/minute)	Average Relative % Humidity	Average Temperature (°C)
Algae stock transfer room air, static plate	up to 0.3	65	23
Algae carboy and small tank culture room, static plate	6.7	65	23
Air conditioner air flow in tank culture room	36	77	23
Tank room carboy air system, air flow	234	77	20
Tank room tank air system, air flow	> 2,000	77	20
Larvae airline, air flow	1500		

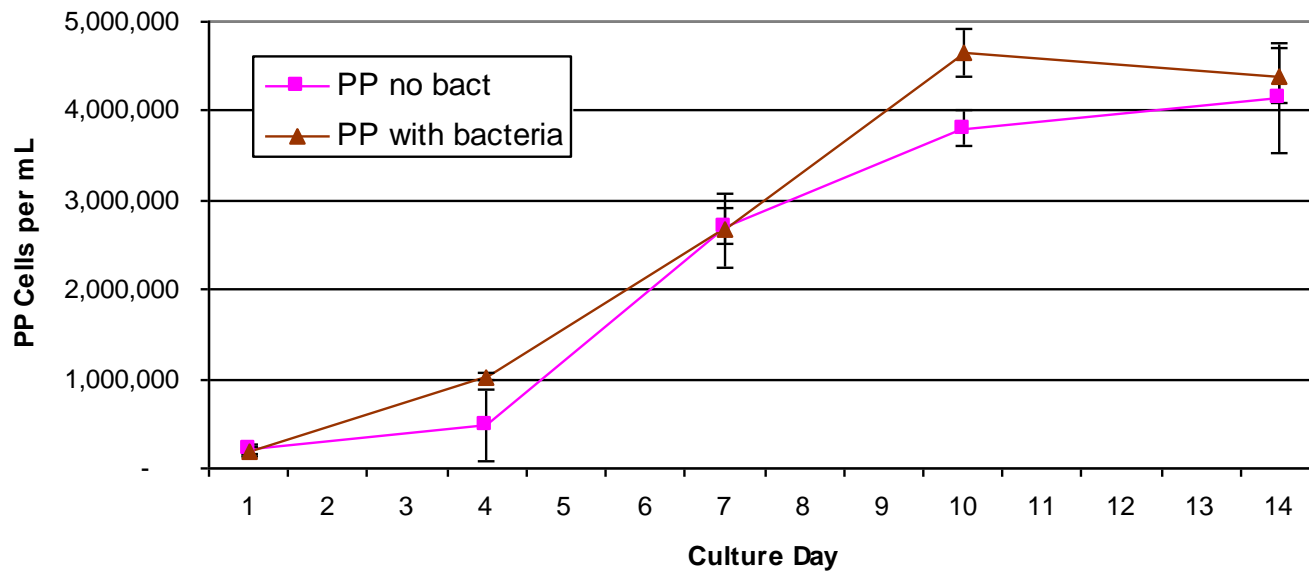
Wetter air = more bacteria

Solutions: drier air and/or air disinfection systems

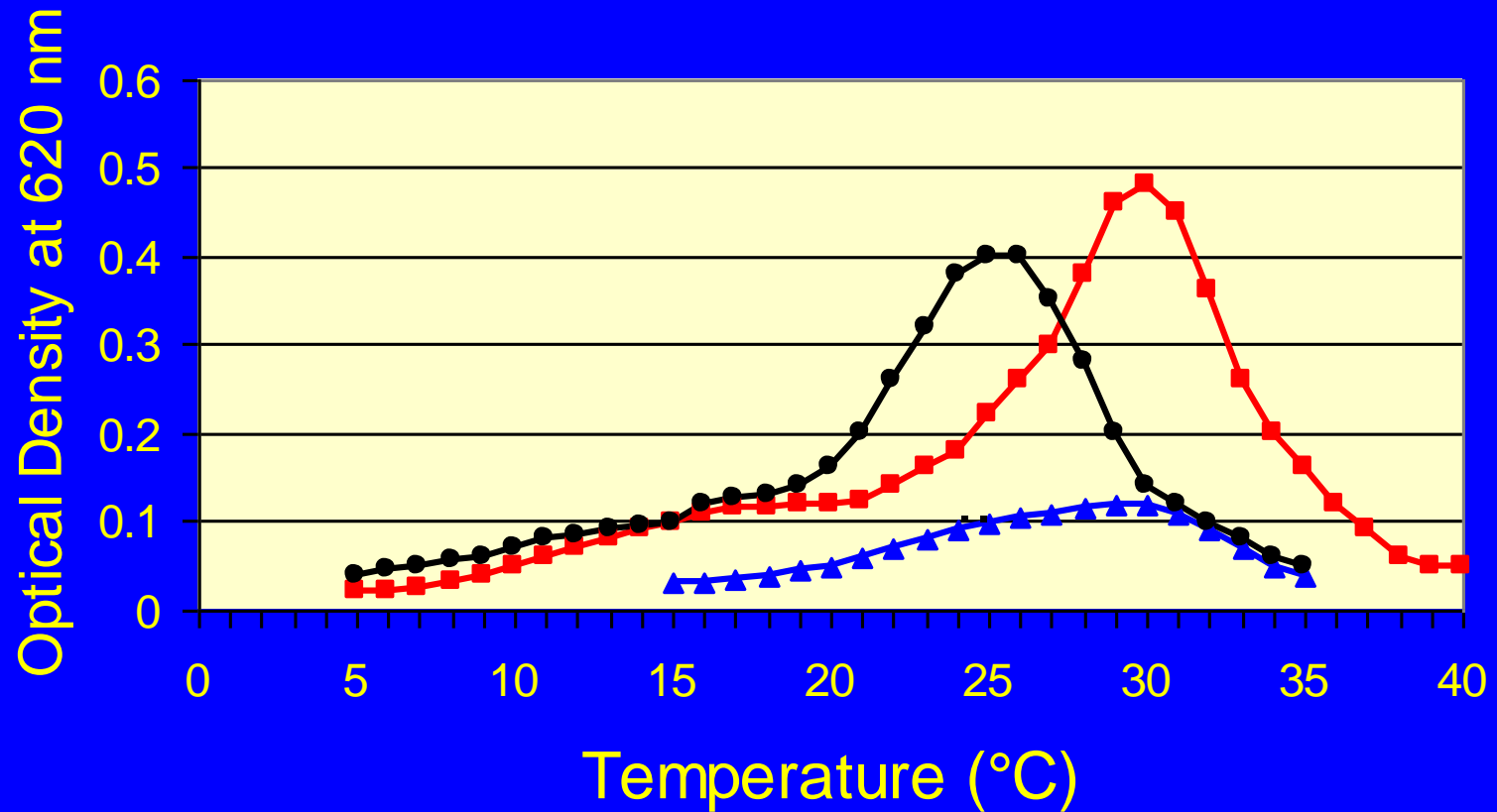
Micro-algae will often co-exist with bacterial contamination – such contamination can even be beneficial for algal but pathogens can also thrive in algal cultures



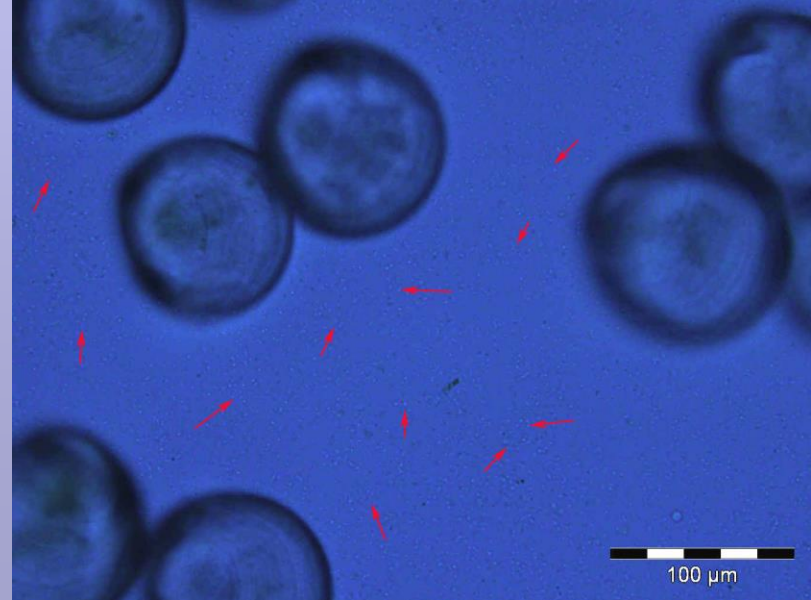
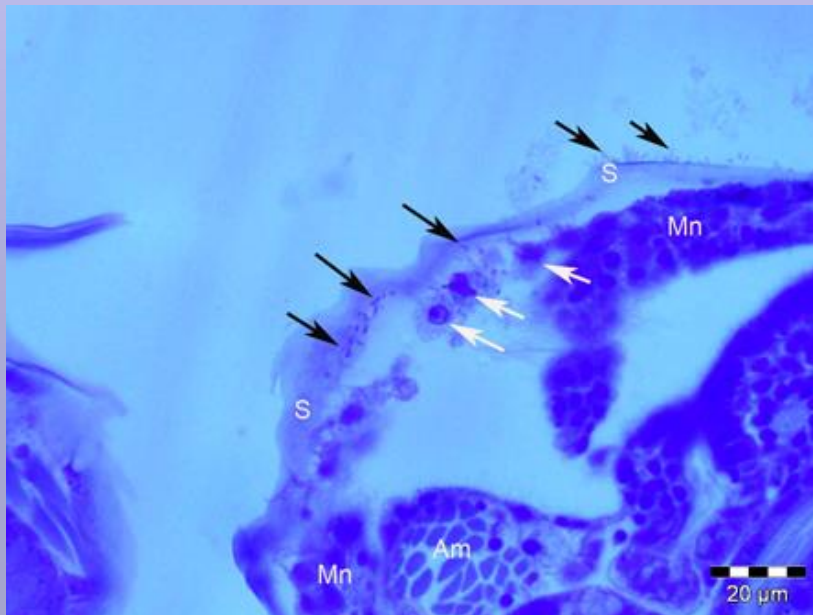
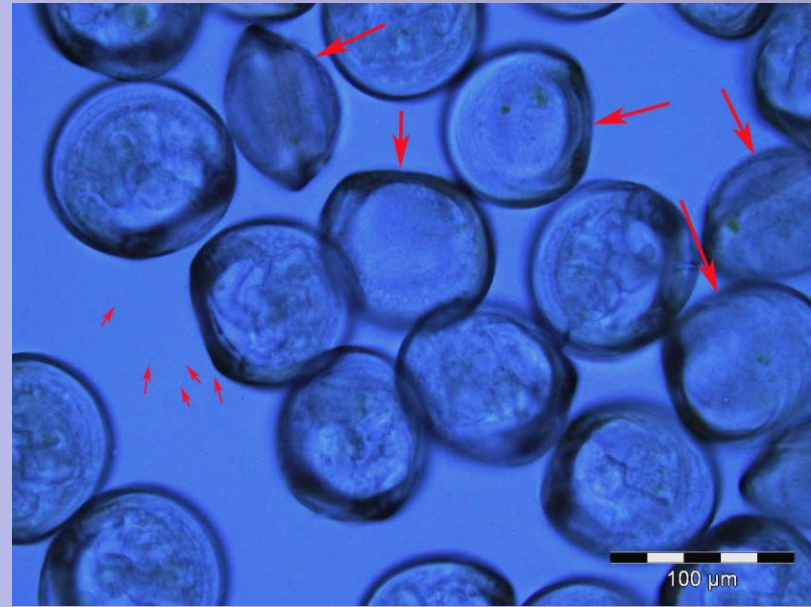
PP Growth with and without 01-68



Temperature - Growth Response of some r-selected bacteria associated with juvenile shellfish morbidity



Vibriosis can be “acute”
(fast acting) or “chronic”
(slower but may be debilitating).....



Pathogenicity of vibrios for larvae and seed is complex and seems to depend on:

- Age of larvae or juveniles and species
- Concentration of pathogenic vibrios (dose) in seawater
- Temperature of seawater
- Growth phase of pathogenic vibrios
- Degree of toxin production by pathogenic vibrios
- Other factors that stress the larvae

Chronic debilitating opportunistic bacterial abscesses in juvenile clams and other bivalves:

Slow growth

Shell deformations

Usually drop out

Resources used to maintain them but either poor survival in nursery or after outplanting

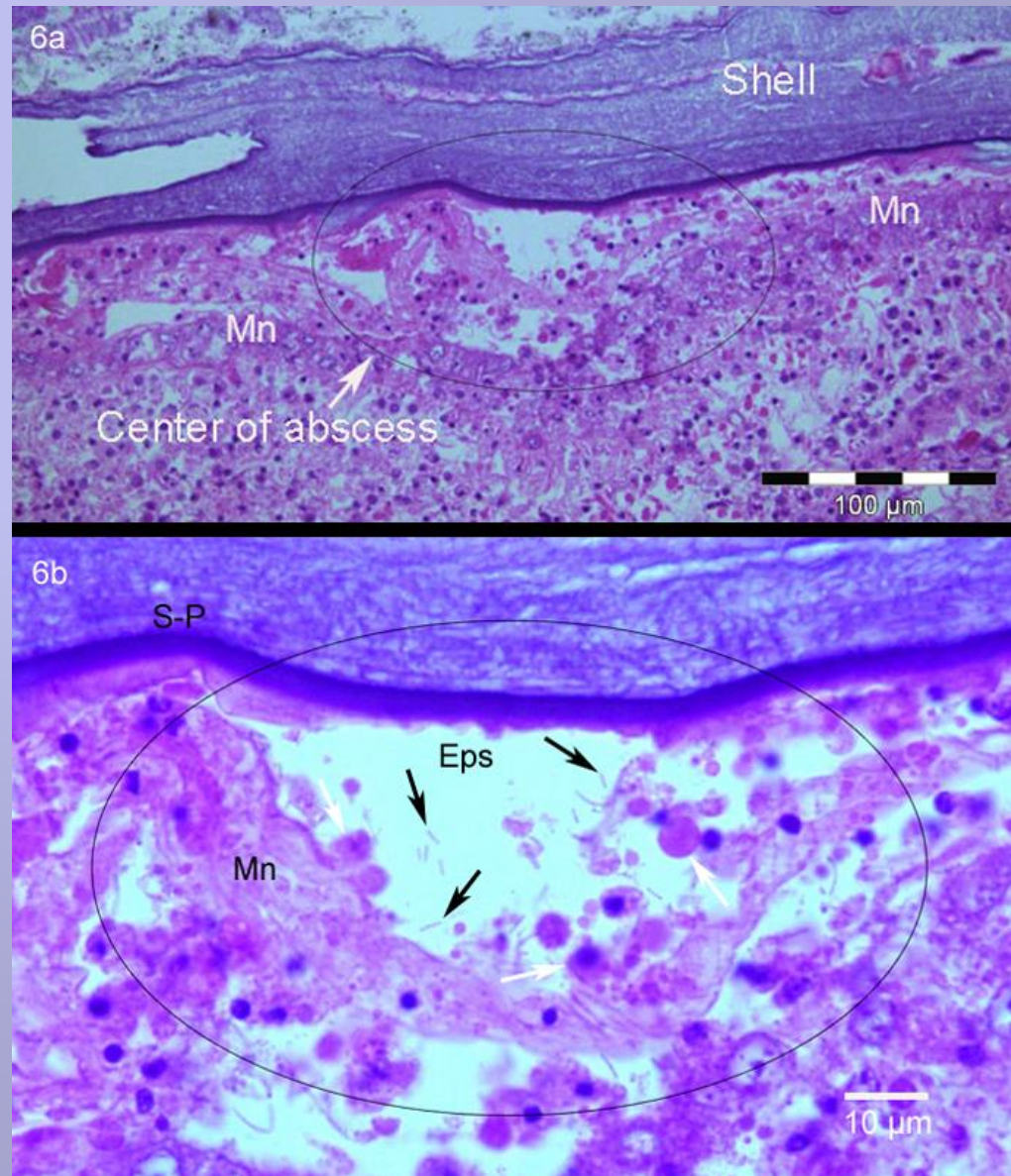
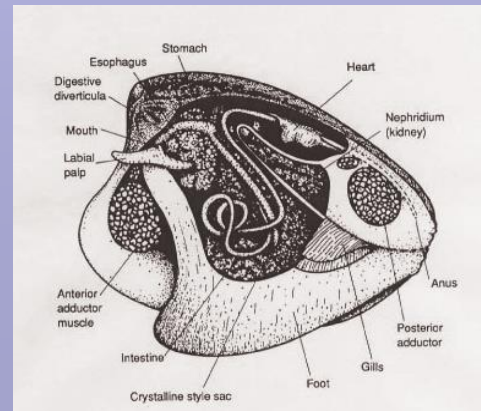
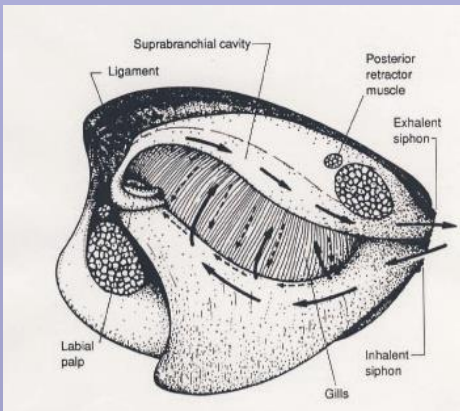
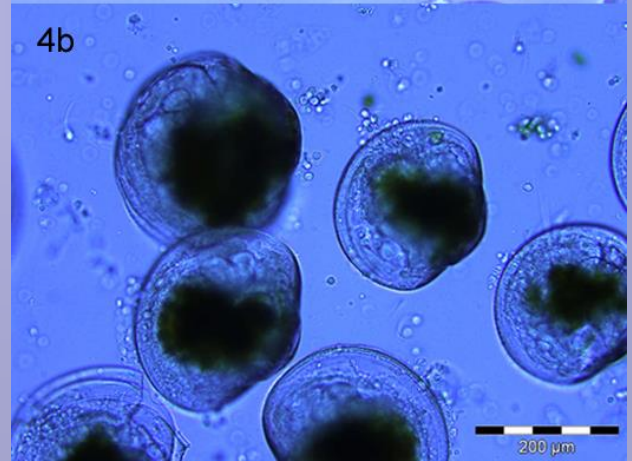
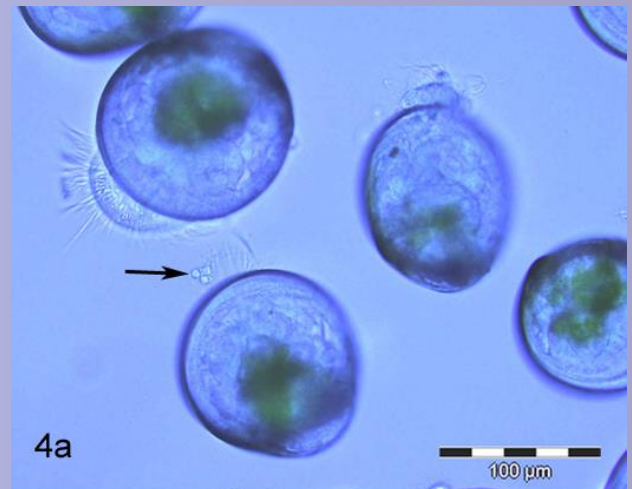
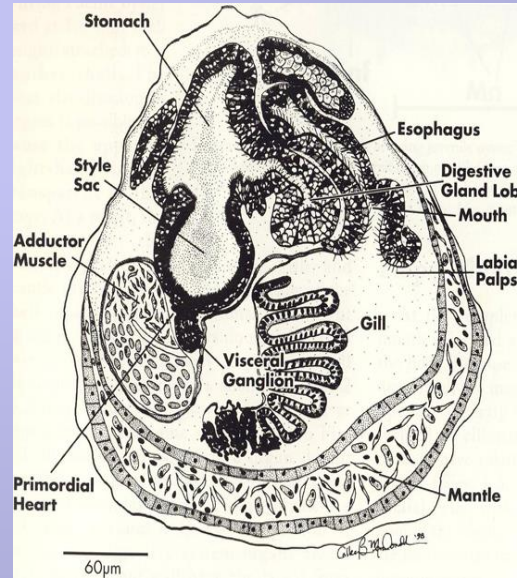
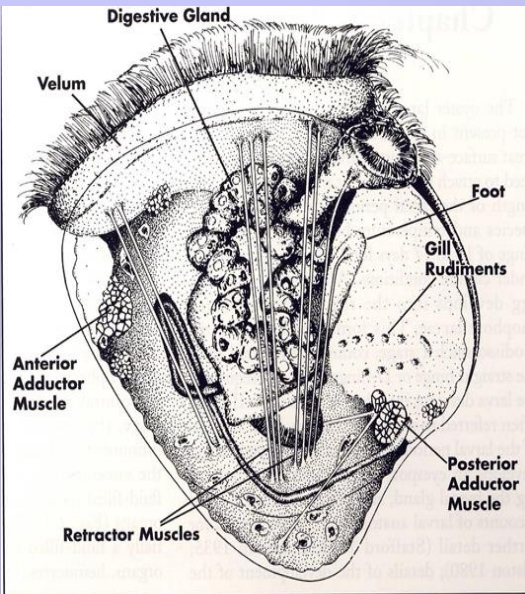


Fig. 6a and 6b. Abscess cause by vibrio bacteria in extrapallial space [Eps] (between mantle and shell) of juvenile – black arrows show bacteria. Mn – mantle; S-P, shell-periostracum.

Understanding anatomy of larvae, juveniles and adults.....



Water quality monitoring and water treatment.....

Measured parameter	Approximate recommended range
Rearing water temperature	Depends on species reared.
pH	7.8 to 8.4 units
Salinity	Depends on species reared
Dissolved oxygen	> 5.0 mg/L, < 5% over saturation
Oxidation reduction potential (ORP)	150-250
Nitrogen cycle	<p><i>Ammonia</i>: Wildly variable; check PAN database for specific organism: 1% of LC50 for target species may be safe. 0.1ppm generally safe. <i>Nitrite</i>: Wildly variable; check PAN database for specific organism: 1% of the LC50 for target species should be relatively safe. .2 ppm should generally be safe.</p> <p><i>Nitrate</i>: Wildly variable; check PAN database for specific organism: Canadian guidelines = 16ppm in SW</p>
Copper	None detectible
Hypochlorite	None detectible
Alkalinity	110-140; few adverse consequences if higher than ca 200 ppmCaCO ₃ . Also measured as dKh (norm 6-7; tolerable to 11) and meq/l (2.1-2.5 = norm)
Total dissolved gas saturation	< 5% greater than saturation

Carbonate – bicarbonate, pH and alkalinity

$$[\text{H}^+] = k \frac{[\text{HCO}_3^-]}{[\text{CO}_3^{2-}]}$$

Adding bicarbonate (HCO_3^-) increases $[\text{H}^+]$ and decreases pH

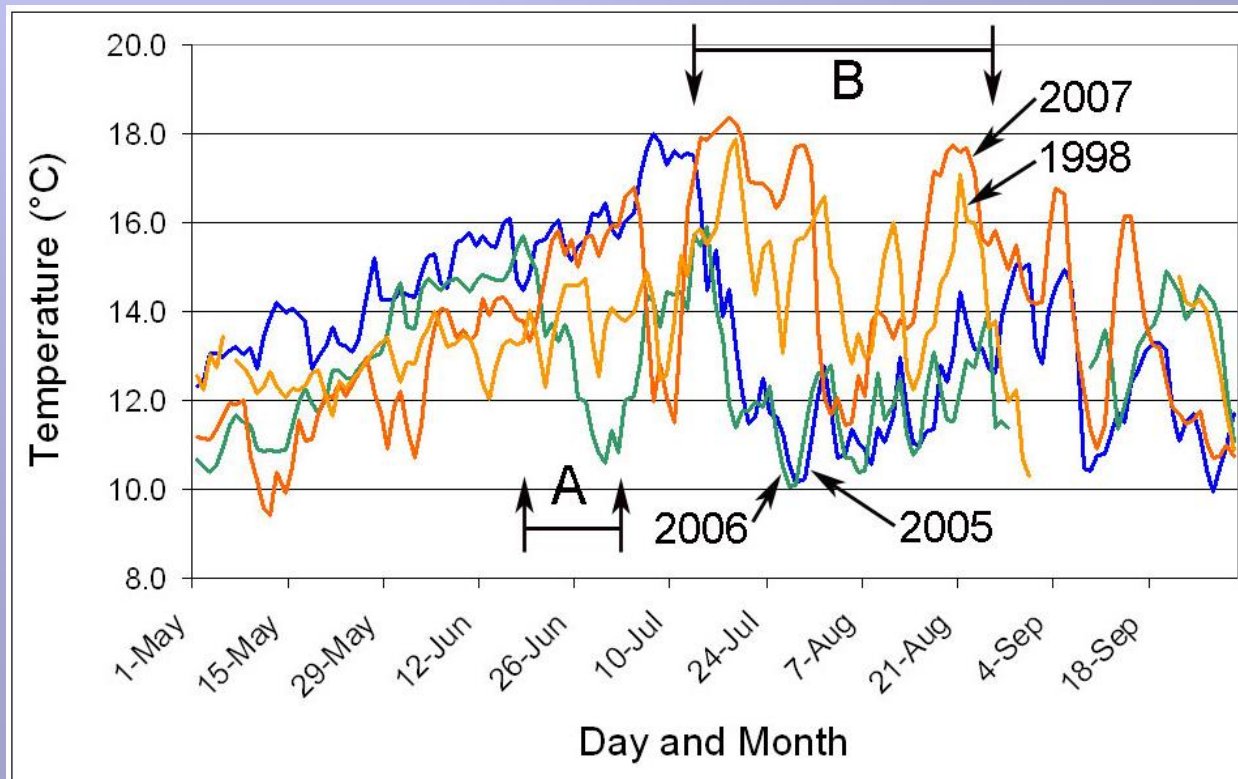
Adding carbonate (CO_3^{2-}) consumes $[\text{H}^+]$ and increases pH

Sodium carbonate (soda ash) may be added to increase alkalinity, and also raises pH

Sodium bi-carbonate (baking soda) may then be needed to reduce pH and further increases alkalinity

Natural temperature cycles in the ocean drive many biological processes, including the concentration of shellfish pathogenic vibrios in the ocean. Example of intermittent upwelling and warm water intrusion combining to cause vibrio outbreak on west coast.

More similar to Atlantic and Gulf coast conditions in Florida may be subtropical experience such as surface water at NELHA Hawaii facility, in which shellfish pathogenic vibrios are present more consistently at low concentrations but are elevated in warmest months of the year.



Water conditioning....

- Well water or sub-sand water, if available and acceptable salinity, for example, may be a good source for algal culture water
- Water treatment – very site specific. Remove zooplankton, large phytoplankton, large debris, may reduce pathogenic bacteria
- Water treatment – can reduce essential trace elements

Water treatment.....

- Multimedia filters – particle size, also remove large concentrations of bacteria that stick to the filter, need to be back flushed with filtered water, not raw seawater
- UV – problematic, because the removed bacterial population must be replaced
- Protein skimmers
- Bioreactors
- Addition of conditioning agents for alkalinity and pH
- Charcoal filtration

Hatchery Management....bacterial pathogens....

- Test and eliminate (reduce), starting with high risk areas
- Requires sustained effort and constant management
- Water filtration and source - water filters need to be cleaned of particulate and large debris and disinfected during periods of high VT input
- Inoculation dose to hatchery may vary with water depth
- Affected spat must be maintained in optimal conditions to clear the infection

.....hatchery and nursery management.....

- Removal of particulate from water removes majority of bacterial cells
- Multimedia filter maintenance during high concentration vibrio episodes – particulates and sterilization
- Toxin removal from water for first 48 hours
- Tightening up the bag filtration system

Pathogenic *Vibrio* “carry over”

- Contamination can carry over to seed
- If seed are too dense, or with poor water flow (particularly singles in floating upwellers)
- Invasive infections may occur and take down large numbers of seed